Study to Determine the Biological Feasibility of a New Fish Tagging System

Annual Report
1984 - 1985

DOE/BP-11982-1

May 1985

This Document should be cited as follows:

Prentice, Earl, Donn Park, Carl Sims, ''Study to Determine the Biological Feasibility of a New Fish Tagging System'', Project No. 1983-31900, 39 electronic pages, (BPA Report DOE/BP-11982-1)

Bonneville Power Administration
P.O. Box 3621
Portland, Oregon 97208

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.

A STUDY TO DETERMINE THE BIOLOGICAL

FEASIBILITY OF A NEW FISH TAGGING SYSTEM

Annual Report of Research

by
Earl F. Prentice
Carl W. Sims
and
Donn L. Park

Funded by
Bonneville Power Administration
U.S. Department of Energy
Division of Fish and Wildlife
P.O. Box 3621
Portland, Oregon 97208
Contract DE-A179-84BP11982, Project 83-319

and

Coastal Zone and Estuarine Studies Division
Northwest and Alaska Fisheries Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
2725 Montlake Boulevard East
Seattle, Washington 98112

May 1985

ABSTRACT

A multi-year cooperative project between the Bonneville Power Administration and the National Marine Fisheries Service was initiated in 1983 to evaluate the potential of the Passive Integrated Transponder (PIT) tag for marking salmonids. The second year's work had three phases. The objective of Phase I was to evaluate fish-tagging with PIT tags. Phases II. and III were concerned with the automatic monitoring of juvenile and adult fish injected with PIT tags.

In Phase I, sham PIT tags were injected into the body cavity of juvenile salmonids. Tagged fish ranged in weight from 0.8 to 43.9 g. Observations based on six tests, ranging in time from 19 to 99 days, indicated that the best site for injecting the tag was along the mid-ventral line in the area of the pectoral or pelvic fins. Potential advantages to tagging the fish in the area of the pectoral fins were noted. As tagging techniques improved, tag retention of 99% ($n = 400$) and survival of 99% were achieved. Minimal tissue response to the tag was noted in both tag locations.

In Phase II, a PIT tag detector system designed to detect and record the passage of juvenile salmonids was evaluated. Detection varied depending upon test conditions. By restricting the rate at which fish entered the monitoring tunnel to one fish per second, detection averaged 94.3% compared to 86.6% for multiple fish entry. Reducing the velocity fish passed through the tag monitoring tunnel from 10 to 8 ft/sec did not affect tag detection.

In Phase III, a monitoring system designed to detect and decode adult fish tagged with a PIT tag was evaluated. The tests were conducted in winter 1984. Adult steelhead and chinook salmon measuring between 39 and 84 cm fork length were used in the test. The fish were tested with functional PIT tags injected into the body cavity. The average detection and proper decoding of these tagged fish was 94.4% for 211 fish. Improvements in the detection system are recommended.

CONTENTS

INTRODUCTION

A multi-year cooperative study between the Bonneville Power Administration and the National Marine Fisheries Service (NMFS) was initiated in 1983 to evaluate the potential of adapting to salmonids a new identification system being developed for livestock. The key element of the identification system is the Passive Integrated Transponder (PIT) tag. The tag presently measures 10 mm long by 2.1 mm in diameter, has about 35 billion unique code combinations, and can be automatically detected and decoded in situ, eliminating the need to anesthetize, handle, or restrain fish during data retrieval.

In 1983-84 juvenile salmonids were injected with sham (nonfunctional) PIT tags in the body cavity and in the opercular, dorsal, and caudal musculature. Adult salmonids were injected in the nose; body cavity; and opercular, dorsal, and caudal musculature with sham PIT tags. Of the anatomical areas evaluated, the body cavity appeared to be the best area for tag placement from a biological and social standpoint. Recommendations were made to continue the biological evaluation of tagging salmonids in the body cavity and to test tag monitoring equipment suitable for automatically detecting and recording PIT tags in juvenile and adult salmonids.

The 1984-85 work had three phases: Phase I, biological evaluation of tagging fish with PIT tags; Phase 11, juvenile salmonid PIT tag monitoring system evaluation; and Phase III, adult salmonid PIT tag monitoring system evaluation. Each phase is discussed separately.

PHASE I: BIOLOGICAL EVALUATION OF TAGGING FISH WITH PIT TAGS

Introduction

The 1983 tests indicated that the body cavity of salmonids was a suitable area to implant the PIT tag. This area was selected after an evaluation of a number of anatomical sites. The following criteria were used in selecting this area: (a) tag retention; (b) effect of the tag on growth, survival, behavior, and wound healing; (c) tissue response to the tag; and (d) social considerations. The 1983 tests suggested that further tests be conducted to develop tagging techniques and to further evaluate the above criteria relative to injecting the tag into the body cavity.

In 1984, tests using functional PIT tags were scheduled to begin in April. However, production delays prevented resting of the functional tag. In place of the planned tests, a series of tests using sham (non-functional) tags were conducted with juvenile steelhead, Salmo gairdneri, and fall chinook salmon, Oncorhynchus tshawytscha. Midway through the testing period, the tag manufacturer changed both the method and material used in encapsulating the electronics of the functional PIT tag. These changes increased the length of the tag from 7.5 to 10 mm and the diameter from 1.5 to 2.1 mm, which required that the injection needle be increased from 14- to 12-gauge.

This report addresses five tests conducted prior to the change in tag dimension (Study A) and a single test conducted after the tag dimension changed (Study B). The objectives of the tests were to determine: (1) the anatomical areas in which the tag could be placed, (2) tissue response to the tag, and (3) tag retention.

Tests using adult salmon, and the design and construction of the hand-operated automatic tag-injection system outlined in the work plan were postponed because of delays in obtaining functional tags.

Methods and Materials

Study A: Tests Performed Prior to Change in Tag Dimensions

In 1984, five tests were conducted prior to substantial changes being made in tag dimensions. All five tests have been combined since the dimension change of the tag, in part, invalidated the results of the five tests. The tests did however provide valuable direction for testing of the new tags. A detailed report on the testing of the new tags is given in Study B of this report.

The five tests were conducted at the University of Washington's Big Beef Creek Research Station. The tests ranged in duration from 19 to 43 days using sham (non functional) tags similar to those used in 1983. The tags were injected into both juvenile steelhead and fall chinook salmon. The number of fish ranged from 25 to 546.

A 14-gauge needle and modified hypodermic syringe were used to inject the tags into fish. All tags were injected into the body cavity. Four anatomical areas for tag injection were evaluated: (1) about 10 mm anterior to the pelvic girdle and about 5 mm lateral to the mid-ventral line; (2) along the mid-ventral line about 5 mm anterior to the pelvic girdle; (3) about 5 mm anterior to the pelvic girdle, just below the lateral line; and (4) along the mid-ventral line at the posterior tip of the pectoral fins. Test fish were held in 4-ft diameter fiberglass tanks receiving a continuous supply of groundwater. Standard husbandry techniques were used to maintain the fish.

Study B: Tests Performed After the Change in Dimensions

The test was conducted from 7 November 1984 to 13 February 1985 at the University of Washington's Big Beef Creek Research Station. Steelhead ranging in fork length from 87 to 154 mm and weight from 4.6 to 43.9 g were divided into nine test groups with 100 fish per replicate (Table 1). A random sample of 10 fish from each group was weighed ($\pm$ 0.1 g) and measured ($\pm$ 1.0 mm), but was not added to the replicate at the start of the study. Additional weight and length information was obtained on 10 fish randomly selected from each replicate on Day 76 and at the termination of the study on Day 99.

The fish were maintained using standard husbandry practices in 24 4-ft diameter circular fiberglass tanks. The tanks received a continuous supply of 10°C groundwater.

Nonfunctional (sham) tags 10.0 mm long by 2.1 mm in diameter were injected into the fish using a modified hypodermic syringe and a 12-gauge needle. The dimensions of the tags were similar to that of the currently available functional tag. Each sham tag consisted of a polypropylene tube, identical to the material used to encapsulate the electronics of the functional tag.

Two body-cavity sites were evaluated. In both areas, the tag injection needle was inserted through the abdominal musculature along the mid-ventral line. In the first area, the needle was angled anteriorly and placed about 5 mm anterior to the pelvic girdle. In the second area, the needle was angled posteriorly and placed at a point on the mid-ventral line that was aligned with the posterior tip of the pectoral fins. In either case, immediately after the needle penetrated the abdominal musculature it was angled to parallel the mid-ventral line and be in contact with the abdominal

Table I.--Summary of 99-day survival and tag retention for steelhead injected with PIT tags.

Treatment	Number of replicates	Number of fish per replicate	Starting number of fish	Recorded mortalities	Number of fish sacrificed	Ending[a] number based on log	Actual[b] ending number	Number of[c] fish missing	Log[d] survival (%)	Count[e] survival (%)	Tags found in tanks	Fish without tags	Tag[f] retention in surviving fish (%)
Control	4	100	400	3	0	397	330	67	99.3	99.1	-		
Needle only pelvic	4	100	400	5	0	395	381	14	98.8	98.7	-		
Needle only pelvic sacrifice		100	100	1	42	57	57	0					
Needle only pectoral	4	100	400	2	0	398	379	19	99.5	99.5	-		
Needle only pectoral sacrifice	1	100	100	0	46	54	33	21					
Pelvic tag	4	100[g]	401	II	0	390	366	24	97.3	97.1	0	5	98.6
Pelvic tag sacrifice	1	100	100	0	42	58	58	0			0	0	
Pectoral tag	4	100	400	3	0	397	371	26	99.3	99.2	1	2	99.2
Pectoral tag sacrifice	1	100	100	1	46	53	50	3		-	3[h]	3[h]	

a/ Starting number minus mortalities and fish sacrificed.
b/ Based on the actual count of fish at the termination of testing.
c/ Number of fish based on log minus actual number of fish present at the termination of testing.
d/ Survival based on the number of fish from log information (excluding sacrificed fish).
e/ Survival based on the number of fish from fish count at termination (excluding sacrificed fish).
f/ Calculations based on actual ending number of fish (count) at the termination of testing minus the number of tags found in the culture tanks and fish without tags.
g/ One replicate had 101 fish at the start of testing.
h/ The higher than usual tag loss can be accounted for by an inexperienced tagger injecting tags into fish of this group.

musculature. The depth of needle penetration depended upon the size of fish being tagged; generally, 5 to 10 mm past the bevel of the needle was sufficient for satisfactory tag placement.

Test groups consisted of controls (no tag or needle) and fish receiving tags (nonfunctional) or sham injections (tagging needle only). Four replicates of 100 fish each were randomly established for the treatments. In addition, one replicate (n = 100) per treatment (excluding the control) (Table 1) was established so that fish could be sequentially sacrificed to observe wound healing, tissue response to the tag, and tag location within the body cavity. On Days 2, 4, 8, 12, 20, 30, and 76, six fish from each sacrificial replicate were killed, visually examined, and scored using a four-point scale that noted the degree to which the wound created by the tagging needle had healed (Table 2). A five-point scale was used to classify the location of the tag within the body cavity (Table 3). All specimens were preserved in buffered 4% formaldehyde for later histological examination.

Initially, all groups were examined for tag loss and mortality every 30 minutes for the first 2 h and daily for the following 9 days. Thereafter, the fish were examined daily except on weekends and holidays. Lost tags were recovered by close examination of the rearing tank bottoms which had specially designed drains to prevent the loss of shed tags. At the termination of the test (Day 99), all tagged fish were sacrificed and examined for the presence of the tag. The tag location within the body cavity and any apparent organ damage that could be associated with the tag were noted. All mortalities that occurred during the test were examined for tag loss and cause of death.

The effect of the tag on growth was analyzed for independence at $P<0.05$ using a Kruskal-Wallis one-way analysis of variance test (Sokal and Rohlf 1981).

Table 2.--Description of the wound classification codes and summary of tagging-wound condition over time.

Treatment	Wound classification code	Day of observation: 0	2	4	6	8	12	20	30	76
		Percent of the fish observed within a wound classification[a/]								
Pelvic tag	A	100	33	0	0	0	0	0	0	0
	B	0	67	100	67	100	100	0	0	0
	C	0	0	0	73	0	0	100	100	0
	D	0	0	0	0	0	0	0	0	100
Pelvic needle only	A	100	0	0	0	0	0	0	0	0
	B	0	100	67	100	100	100	17	0	0
	C	0	0	33	0	0		83	100	0
	D	0	0	0	0	0	0	0	0	100
Pectoral tag	A	100	0	0	0	0	0	17	0	0
	B	0	100	67	83	100	100	17	0	0
	C	0	0	33	17	0	0	66	100	0
	D	0	0	0	0	0	0	0	0	100
Pectoral needle only	A	100	0	0	0	0	0	0	0	0
	B	0	100	83	33	83	83	0	0	0
	C	0	0	17	67	17	17	100	100	0
	D	0	0	0	0	0	0	0	0	100

Wound Classification Code Description

Wound Code A - an open wound.

Wound Code B - a wound that is closed by a thin membrane and is healing. At times a slight red or pinkish coloration is noticeable in the area of the wound.

Wound Code C - a wound completely healed and noticeable only by the presence of a scar. There is no red or pink coloration in the area of the wound.

Wound Code D - a wound completely healed and only noticeable after careful examination. Little or no scar tissue.

a/ Observations based on six fish per observation period.

Table 3.--Description of the tag location code and summary of tag location within the body cavity of juvenile steelhead over time.

		Days																	
		2		4		6		8		12		20		33		76		99	
Treatment	Tag location	Number of tags	%	Number of tags	%	Number of tags	%	Number of tags	%	Number of tags	%	Number of tags	%	Number of tags	%	Number of tags	%	Number of tags	%
Pectoral tag serial sacrifice	A	2	34	0	0	1	17	0	0	1	17	0	0	0	0	5	10	-	-
	B	0	0	3	50	4	68	1	17	0	0	1	17	2	33	1	2	-	-
	C	3	51	3	50	1	17	4	68	5	85	4	68	3	50	36	72	-	-
	D	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-	-
	E	1	17	0	0	0	0	1	17	0	0	1	17	1	17	8	16	-	-
Pectoral tag (four replicates combined)	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	1
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	86
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	320	86
	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	2
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1
Pelvic tag serial sacrifice	A	1	17	0	0	1	17	4	66	[a]	-	0	0	1	17	18	31	-	-
	B	2	33	1	17	2	33	0	0	[a]	-	4	66	2	33	4	7	-	-
	C	3	50	4	66	3	50	2	33	[a]	-	2	33	2	33	35	60	-	-
	D	0	0	1	17	0	0	0	0	[a]	-	0	0	1	17	1	2	-	-
	E	0	0	0	0	0	0	0	0	[a]	-	0	0	0	0	0	0	-	-
Pelvic tag (four replicates combined)	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90	25
	B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	209	57
	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	62	17
	D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	0
	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	1

Tag Location Code Description:

A = Tag located between the pyloric caeca and mid-gut.
B = Tag embedded in the pyloric caeca generally near the spleen.
C = Tag located near the abdominal musculature near the mid-ventral line. The tag was often embedded in the posterior area of the pyloric caeca or in the adipose tissue at the posterior area of the pyloric caeca.
D = Tag found in an area other than those noted; generally between the mid-gut and air bladder or between the liver and pyloric caeca.
E = No tag present.

[a] Sample not processed because of improper preservation.

Results and Discussion

Study A

Tag retention and fish survival were influenced by tagging technique. Best results occurred when the point of the hypodermic needle was placed at an approximate 45 degree angle to the body surface of the fish with the needle's bevel away from the body. A slight twisting action while exerting pressure on the tag injection system aided in penetration and displacement of small scales, thus reducing the pressure needed to penetrate the body wall of the fish. Once the needle entered the body cavity, the needle angle was immediately changed so that the beveled portion of the needle was in contact with the inner surface of the body wall. If the needle angle was not altered, vital organs were occasionally perforated. In all cases, a perforated intestine or spleen resulted in death within 4 to 5 days. In many cases, the fish's color would darken within an hour after tagging and the fish would show erratic swimming behavior. Depth of the needle penetration, depending on fish size, was approximately 5 to 10 mm beyond the bevel of the needle. If needle insertion was too shallow, the tag had a tendency to be forced out the open wound from pressure exerted by muscles or internal organs.

Tests showed that slight changes in the area through which the tag was injected into the body cavity could affect survival and tag retention. Initially, the tag was injected into the body cavity by penetrating the body wall about 10 mm anterior to the pelvic girdle and about 5 mm lateral to the mid-ventral line, with the tagging needle directed anteriorly. This site was eventually modified so that penetration occurred along the mid-ventral line about 5 mm anterior to the pelvic girdle with the tagging needle pointed anteriorly. This site was preferable to the previous site in that the incidence of penetrating the intestine or spleen was reduced. A third site was about 5 mm anterior to the pelvic girdle, just below the lateral line. The tagging needle was pointed anteriorly. Tag retention was found to be 98% after 19 days, however a 20% mortality resulted from perforation of the intestine by the tagging needle. In addition, several tags were found within the air bladder of live fish. In these cases the air bladder was completely inflated and showed no damage upon visual examination. A fourth site for injecting the tag into the body cavity was evaluated, again along the mid-ventral line but at the posterior tip of the pectoral fins, with the tagging needle pointed posteriorly. The advantage of this site over others was that the pyloric caeca and gut did not exert pressure on the tag to force it out of the tagging wound prior to healing.

Tests showed that the PIT tag could be successfully injected into fish weighing 0.8 g. It was concluded, however, that salmonids weighing less than approximately 3 to 4 g and measuring less than 65 to 75 mm fork length should normally not be tagged without special handling procedures. Tagging fish less than 3 to 4 g substantially increases the risk of perforating the intestine with the tagging needle because of the small size of the body cavity. Very small fish also showed behavioral changes lasting several days after tagging.

Tn conclusion, evaluation of the five tests resulted in refinements in tagging technique, indications that placement of the tag within the body cavity was the most acceptable technique, and a better understanding of the minimum size fish that can be injected with the PIT tag. Additionally, the tests clarified the tag rejection process and tissue response to the tag. These tests also provided valuable insight in the design and execution of Study B.

Study B

If severe problems had resulted from the tagging operation or the presence of the tag within the fish, there should have been a noticeable depression in growth in relation to the control group. However, during the 99 days of testing, no statistically significant differences ($P<0.05$) were seen in length or weight among the various test groups (Table 4) with the exception of the group receiving sham injections in the pelvic area. This group was significantly shorter ($P<0.05$) compared to the other test groups on Day 99. No explanation for this can be offered since the Pelvic Tag Group did not show a comparable difference in length.

Survival and tag retention are dependent upon the tagging procedure. In this study, fish were not fed for 2 days before or after the test started. This fasting period allowed time for food to clear the stomach and gut and for the tagging wound to partially heal before feeding. An empty stomach and gut is believed to reduce stress during tagging by reducing the size of these organs, thus decreasing the likelihood of perforation with the tagging needle. Also, forcing of the tag back through the tagging wound prior to healing may be lessened by reducing the pressure that could be exerted on the tag by an organ. No tests have been conducted to verify the apparent advantage of not feeding the fish before and after tagging, however, 2 years of observations suggest that this procedure is sound.

The unexpected loss of fish to river otters complicated the calculations for survival. The otters entered the rearing tanks by climbing under bird covers secured on the top of each tank. Since accurate mortality records were maintained for each tank, and since little mortality occurred in any of the treatments, survival data are presented both on daily log information and

Table 4.--Summary of growth data by treatment at three observations periods.

Treatment	Day 0 Mean a/ length (mm)	Day 0 Sd	Day 0 Mean a/ weight (g)	Day 0 Sd	Day 76 Mean length (mm)	Day 76 Sd	Day 76 Mean weight (g)	Day 76 Sd	Day 99 Mean length (mm)	Day 99 Sd	Day 99 Mean weight (mm)	Day 99 Sd
Pelvic tag	116.6	15.5	19.3	8.0	126.6	21.4	23.4	10.9	144.2	20.6	36.3	12.8
Pelvic needle only	116.3	18.1	19.5	a.7	126.1	14.3	21.3	8.3	133.3 b/	14.4	27.1	8.9
Pectoral tag	114.6	13.1	18.5	6.2	132.1	12.8	24.3	7.7	139.7	19.1	32.0	12.0
Pectoral needle only	116.1	14.0	19.0	6.8	130.8	14.1	25.2	9.2	141.9	15.7	33.4	10.8
Control	112.4	16.2	17.2	7.3	128.2	18.0	24.1	10.8	141.1	19.1	32.9	13.5

a/ Mean of 10 fish from each of the five replicates (n = 50) except for the control which had four replicates (n = 40).

b/ Significant difference ($F_{4,15}$ = 3.62, P < 0.05).

actual count of fish at termination (Table 1). The unaccounted fish loss is listed for each treatment in Table 1.

Survival was high and there were no major differences in survival between any of the test groups (Table 4). There was, however, a trend for both the Pelvic Tag and Pelvic Needle-Only Groups to have a slightly higher mortality than the other test groups.

In the study, 7 fish (0.35%) out of 2,001 injected with the tagging needle died due to a perforated gut or organ damage. Five of the seven fish were from the Pelvic Tag and Pelvic Needle-Only Groups. All fish that succumbed due to perforated organs showed color change (darkening) and/or behavioral changes immediately after needle injection. All seven fish died within 3 days after tagging. Similar results have been observed in other tests. Deaths from a perforated gut or organ damage normally occur within 4 to 5 days.

The slightly higher mortality rate associated with the pelvic tag location was thought to be related to the angle of the tagging needle after it entered the body cavity. If care was taken to angle the needle along the abdominal musculature and align it parallel to the mid-ventral line immediately after entering the body cavity, perforation of the gut was avoided.

The time for the tagging wound to close and heal is important for two reasons. First, an open wound increases the possibility of disease or infection. Secondly, the likelihood of the tag being expelled from an open wound is much higher than from a healed wound.

A subsample of six fish was randomly removed from one sacrificial replicate (n = 100 fish) for each treatment group, with the exception of the

Control Group, on Days 2, 4, 6, 8, 12, 20, 30, and 76 so that visual and histological observations could be made on tag location, tagging wound condition, and tissue response. The tagging wound conditions for each treatment group on the above observation dates are presented in Table 2. By Day 2, nearly all the wounds were closed by a thin membrane (Wound Code B). Some of the wounds showed slight reddening and pink coloration. Within 6 days of tagging, a number of the wounds appeared to be completely healed (Wound Code C) with only a scar showing where the tagging needle had been inserted into the body musculature. Between Pays 6 and 12, there was a period where the wound condition deteriorated or was stable (Wound Code B). By Day 20, nearly all the wounds appeared healed, and by Day 76 it became difficult to locate where the tagging needle had penetrated the abdominal musculature. No differences in rate of wound healing were seen between the various treatments. The observed suppression in wound healing between Days 6 and 12 can not be explained, however, it did not affect tag retention or survival, which was nearly 100% in all groups. Based on the results of this and other tests, we believe that by the eighth or twelfth day after tagging the wound has healed sufficiently to prevent both infection and tag loss. It should be noted that all tests have been conducted using groundwater that was relatively pathogen free. It is recommended that a test be conducted using surface water to verify these results.

Tag loss within 48 h after tagging had been a problem in previous tests, at times reaching 14%. Tags which were subsequently lost have been observed protruding from tagging wounds. In the present study, observations were made to document the period of highest tag loss. No tag loss was recorded within the first 15 days of the test among the eight test replicates (four pelvic tag and four pectoral tag replicates). However, three tags were shed within the Pectoral Sacrificial Group during the first 3 days of study. A person not experienced with injecting the tag into the body cavity of fish had assisted in tagging the Pectoral Sacrificial Group. The tagging needle had not been inserted into the body cavity at the same angle and depth as previously described. We believe this change in technique accounted for the initial tag loss in this group of fish. The high tag retention in all tag groups was, in part, aided by starving the fish 2 days before and 2 days after the test started for reasons previously cited.

Few tags were lost during the 99 days of testing (Table 1). No major differences in tag retention were observed within or between treatments. In the Pectoral Group, one tag was found in the culture tank and two fish were found without tags at the termination of testing (99.2% tag retention). Tag loss in the Pelvic Group consisted of one tag not present in a dead fish and four tags not found in fish at the termination of testing (98.6% tag retention). These calculations are based on the original number of fish (excluding the sacrificial groups) with no adjustment for unaccounted fish loss. We believe this is justified due to the low overall tag loss in relation to the large number of fish used. Making adjustments for the unaccounted fish loss does not significantly alter the results (Table 1).

We believe there are some advantages to tagging juvenile fish in the pectoral position with the tagging needle directed posteriorly. After injection, the tag normally lies between the abdominal wall and pyloric caeca or in the posterior portion of the pyloric caeca. In these positions, the tag, if it is to be shed, must be forced into the pyloric caeca and align itself with the tagging wound; this is less likely than if the tag had been inserted via the pelvic are , with the tagging needle directed anteriorly. Secondly, there is less chance of penetrating the gut or spleen by injecting the tag via the pectoral position, since the tagging needle is directed away from vital organs. These apparent advantages may only apply to juvenile fish since adult fish have well-developed scales; it may require less effort to penetrate the abdominal. wall. by injecting the tag via the pelvic area as noted. Because there is substantially more area within the body cavity of an adult fish, the risk of penetrating a vital organ is reduced.

Tissue response to the tagging needle was limited. Insertion of the needle and tag through the abdominal musculature elicited an initial acute inflammatory response with little or no hemorrhage or edema. Most tags were found imbedded within the mesentery with little or no adverse tissue reaction to the tag. Melanomacrophages were observed in the area of about 50% of the tags examined at the termination of testing. This deposition of melanin is a normal response to a foreign body within tissue and is not considered detrimental.

Location of the tag within the body cavity was consistent with the area of insertion (pectoral or pelvic) (Table 3). This observation suggests that once the tag was injected into a juvenile fish, the tag did not migrate substantially. The majority of the tags (86%) in the Pectoral Group were located near the abdominal musculature along the mid-ventral line at the termination of the test (Day 99). The tag was often imbedded in the posterior area of the pyloric caeca or in the adipose tissue at the posterior end of the pyloric caeca. This is in contrast to only 17% of the tags found in this location in the Pelvic Group. The majority of the tags (57%) in the Pelvic Group were found imbedded in the pyloric caeca near the spleen, whereas only

10% of the tags in the Pectoral Group were found in this location. A second area for high numbers of tags in the Pelvic Group was between the mid-gut and pyloric caeca (25%). The difference in tag location between the two treatment groups reflects the differences in direction and angle that the tags were injected into the fish. There is potential physical damage to juvenile fish by injecting the tag via the pelvic location.

Conclusions and Recommendations

1. Based on data for survival, tag retention, and tissue response, the PIT tag can be injected successfully and retained in the body cavity of juvenile steelhead weighing from 4.6 to 43.9 g.

2. Placement of the tag via either the pectoral or pelvic position is satisfactory, however, it is recommended that the tag be inserted via the pectoral position in juvenile fish because of the final tag location within the body cavity, the reduced possibility of puncturing an organ, and the reduced possibility of shedding the tag immediately after tagging.

3. The tag did not affect survival in any of the test groups.

4. The tag did not significantly affect growth in any of the tagged test groups.

5. Tag retention was not markedly different between treatments, however, there was a trend for the pectoral site to have a slightly higher retention.

6. The tagging wounds appeared to be closed sufficiently to prevent tag loss or wound infection 8 to 12 days after tagging, Since all tests were conducted in groundwater that was relatively pathogen free, it is recommended that all further tests be conducted using surface water to verify the results.

7. After tagging, if the gut was perforated by the tagging needle, there was an immediate change in fish color and/or behavior. Death usually occurred within 4 to 5 days.

8. If tags were lost from a fish, the loss normally occurred within 3 to 4 days after tagging.

9. It is recommended that tagging procedures be developed that reduce the possibility of disease transmission through the tagging equipment. To date, no such disease transmission has been seen, however, there is a potential for such a problem.

10. No long-term behavioral difference was observed between tagged and untagged fish, but it is recommended that this observation be verified by a series of tests.

11. A long-term test (juvenile to maturity) is recommended using functional tags to verify all results. Such a test would not only provide valuable biological information but would provide information concerning the reliability and longevity of the tag.

12. We recommend that until additional laboratory and field tests are conducted and the data analysed, that a cautious approach be taken in the use of the PIT tag even though all the information to date is encouraging. Premature use of the tag may give biased results stemming from a lack of understanding of the technical limitations of the tag and monitoring system, and an incomplete understanding of the biological ramifications of injecting the tag into fish. We believe that if test results continue to be as encouraging as they are, that a field test be conducted in spring 1986.

PHASE II: MONITORING OF PIT TAGS IN JUVENILE FISH

Introduction

The PIT tag offers biologists studying juvenile salmonids a potentially effective research tool. If the tag and detectors function according to design criteria, accurate assessments of smolt travel time, migration timing, and survival would be possible by tagging relatively small numbers of fish without the need of handling untagged fish at the recapture points. In 1984, NMFS contracted to assess and evaluate a PIT tag detector system designed to automatically detect and record the passage of juvenile salmonids. The objectives of this testing program were to: (1) measure the accuracy and reliability of the PIT tag detector and (2) assess the feasibility of installing and operating such detectors at the fingerling collector dams on the Snake and Columbia Rivers.

Methods and Materials

The prototype PIT tag detector system provided by Identification Devices Inc. (Fig. 1) consisted of four detector loops (two vertical and two oblique) wrapped around a 48-inch long rectangular fiberglass tunnel (6 x 12 inches, inside dimensions). Each detector loop was attached to a common loop excitor assembly that was in turn connected to a single controller assembly (programmer) and a recorder (Fig. 1). Multiple detector loops and different loop orientations were used to increase the probability of tag detection given various tag orientations within fish.

Tests were conducted at the NMFS' oval-flume test facility in Pasco, Washington, during winter 1984 to assess the accuracy and reliability of this system. Replicate groups of juvenile fall chinook salmon (140-250 mm fork

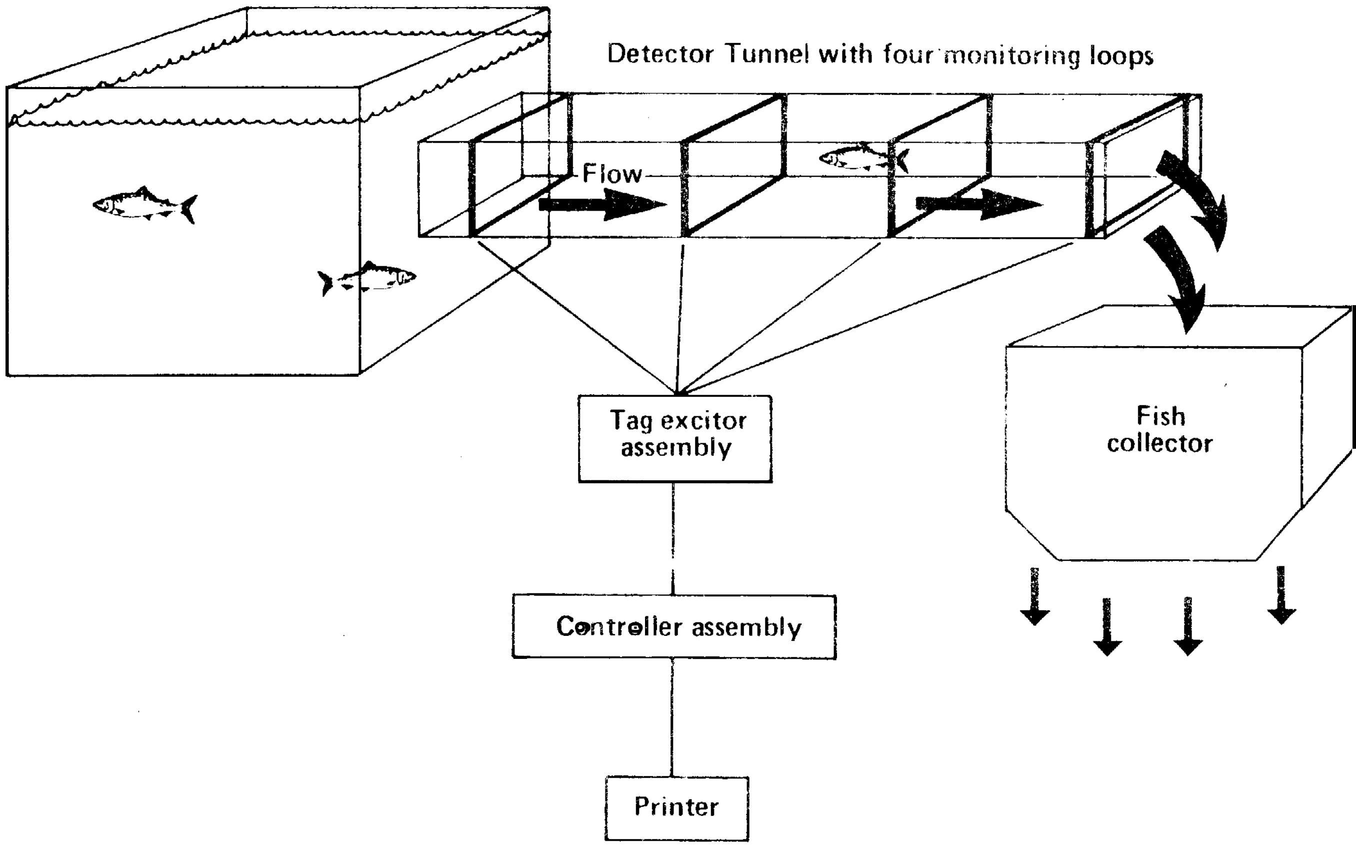

Figure 1.--Diagram of PIT tag detection system.

length) were run through the detector tunnel. Test groups were introduced into the detector tunnel in two ways: at l-second intervals or all at the same time. Both conditions were tested at 8 and and 10 ft/sec water velocity to simulate the velocity ranges in the separator flume at the McNary Dam collection facility.

Test fish were tagged by inserting the functional PIT tag into the body cavity using a modified hypodermic needle with the method described in Phase I of this report. After tagging, a hand-held tag detector was used to record tag numbers and to verify that each tag was functional.

Results and Discussion

Four tests were conducted to assess the prototype PIT tag detection system (Table 5). Results of these tests are summarized in Table 6. Percent detection for the four tests averaged 90.5%. When test fish were introduced into the detection tunnel at l-second intervals (Tests 1 and 3), percent detection was 94.5% at 10 ft/sec tunnel velocity and 94.0% at 8 ft/sec. When multiple tunnel entry was tested (Tests 2 and 4), percent detection fell to 85.8 and 87.5% at 8 and 10 ft/sec. The difference in detection between 8 and 10 ft/sec was not statistically significant for either individual or multiple tunnel entry (Table 7). At the two velocities tested, the detection associated with individual tunnel entry was not significantly higher than that associated with multiple tunnel entry [85.8 to 94.0% at 8 ft/sec and 87.5 to 94.5% at 10 ft/sec (Table 8)]. Since the probability of more than one PIT tagged fish entering a detection tunnel at the same time at a collector dam would be very low, we can assume the accuracy of an operational system in place at a collector dam should approach 94% even if no further improvements to the prototype system are forthcoming. This level of accuracy exceeds that

Table 5.--PIT tag detection tests - 1984.

Test 1 7 November 1984

Number of fish: 110

Velocity: 10 ft/sec Type of passage: Individual[a]

Replicate number	Tag number 36B	511	607	934	649	83E	967	A23	D45	C69	E04	Number detected	Percent detected
1	X[b]	X			X	X	X	X	X	X	X	9	81.8
2	X	X	X	X	X	X	X	X	X	X	X	11	100.0
3		X	X	X	X	X	X	X	X	X	X	10	90.9
4		X	X	X	X	X	X	X	X	X	X	10	90.9
5	X	X	X	X	X	X	X	X	X	X	X	11	100.0
6	X	X	X	X	X	X	X	X	X	X	X	11	100.0
7	X	X	X	X	X	X	X	X	X	X	X	11	100.0
8	X	X	X	X	X	X	X	X	X		X	10	90.9
9	X	X	X	X	X	X	X	X	X	X	X	11	100.0
10	X	X	X	X	X	X	X	X	X		X	10	90.9

Mean detection rate = 104/110 - 94.5%

Test 2 14 November 1984

Number of fish: 120

Velocity: 8 ft/sec Type of passage: Multiple[c]

Replicate number	Tag number 126	F44	42C	967	649	35E	D45	C28	902	920	A23	511	Number detected	Percent detected
1	X	X	X	X	X	X	X	X	X		X	X	11	91.7
2	X	X	X	X	X	X	X	X	X	X	X	X	12	100.0
3	X	X		X		X	X	X	X	X	X	X	10	83.3
4	X	X		X	X	X	X	X		X	X	X	10	83.3
5	X		X	X		X	X	X	X	X	X	X	10	83.3
6	X	X	X	X	X	X	X	X	X		X	X	11	91.7
7	X	X	X		X		X	X	X	X	X	X	10	83.3
8	X	X	X	X	X	X	X	X	X	X		X	11	91.7
9	X			X	X		X	X	X		X	X	8	66.7
10		X	X	X	X	X	X		X	X	X	X	10	83.3

Mean detection rate = 103/120 = 85.8%

Table 5.--cont.

Test 3 15 November 1984

Velocity: 8 ft/sec Number of fish: 100
Type of passage: Individual

Replicate number	862	F44	COB	967	C28	35E	D45	920	A23	511	Number detected	Percent detected
1	x	x	x	x	x		x	x	x	x	9	90.0
2	x	x	x	x	x	x	x	x	x	x	10	100.0
3		x	x	x	x	x	x	x	x	x	9	90.0
4	x	x	x	x	x	x	x	x	x	x	10	100.0
5	x	x	x	x	x	x	x	x	x	x	10	100.0
6	x	x	x	x	x	x	x	x	x	x	10	100.0
7	x	x	x	x	x	x	x	x	x	x	10	100.0
8	x	x		x	x	x	x		x	x	8	80.0
9	x	x			x	x	x	x	x	x	8	80.0
10	x	x	x	x	x	x	x	x	x	x	10	100.0

Mean detection rate = 94/100 = 94.0%

Test 4 29 November 1984

Velocity: 10 ft/sec Number of fish: 120
Type of passage: Multiple

Replicate number	126	F44	42C	967	649	35E	D45	C28	902	920	A23	511	Number detected	Percent detected
1	x	x	x	x	x	x	x		X		x	x	10	83.3
2	x	x	x	x		x	x	x	x	x	x	x	11	91.7
3	X		x	x	x	x	x	x	x	x	x	x	11	91.7
4	x	x	x	x	x	x	x	x	x	x	x	x	12	100.0
5	X	x	x	x	x		x	x	x	x	x		10	83.3
6	x	x	x	x			X		X			X	7	58.3
7	x	x	x	x	x	x	x	x		x	x	x	11	91.7
8	x	x		x	x	x	x	x	x	x	x	x	11	91.7
9	X	x	x	x	x	x	x	x	x	x	x	x	12	100.0
10	x	x	x		X		x	x	x	x	x	x	10	83.3

Mean detection rate = 105/120 = 87.5%

a/ Test fish introduced into detector tunnel at l-second intervals.
b/ Denotes tag detected.
c/ Test fish introduced into detector tunnel a11 at one time.

Table 6.--Summary of PIT tag detection tests conducted at Pasco, Washington, 1984.

	Velocity (ft/sec)	Type of entry	Number fish tested	Number detected	Detection (%)	95% confidence interval
Test 1	10	individual[a]	110	104	94.5	90.0-99.1
Test 2	8	multiple[b]	120	103	85.8	79.5-92.1
Test 3	8	individual	100	94	94.0	87.9-100.0
Test 4	10	multiple	120	105	87.5	78.9-96.0

a/ Test fish introduced into detector tunnel at 1-second intervals.

b/ All test fish within each replicate introduced into detector tunnel at same time.

Table 7.--Contingency table test for the effects of tunnel velocity on PIT tag detection.

Individual tunnel entry

Velocity	Number detected	Number not detected	Total
8 ft/sec	94	6	100
10 ft/sec	104	6	110
Totals	198	12	210

$\chi^2 = 0.016$

Since $\chi^2_{0.05\ 1\ df} = 3.841$ difference not significant at $P = 0.05$

Multiple tunnel entry

Velocity	Number detected	Number not detected	Total
8 ft/sec	103	17	120
10 ft/sec	105	15	120
Totals	208	32	240

$\chi^2 = 0.36$

Since $\chi^4_{0.05\ 1\ df} = 3.841$ difference is not significant at $P = 0.05$

Table 8.--Contingency table test for effects of multiple tunnel entry on PIT tag detection.

8 ft/sec velocity

Tunnel entry	Number detected	Number not detected	Total
Individual	94	6	100
Multiple	103	17	120
Totals	197	23	220

$\chi^2 = 3.063$

Since $\chi^2_{0.05\ 1\ df} = 3.841$ the difference is not significant at P = 0.05.

10 ft/sec velocity

Tunnel entry	Number detected	Number not detected	Total
Individual	104	6	110
Multiple	105	15	120
Totals	209	21	230

$\chi^2 = 2.637$

Since $\chi^2_{0.05\ 1\ df} = 3.841$ the difference is not significant at P = 0.05.

of the freeze-brand marking system now used for most juvenile salmonid marking experiments.

The installation and testing of the detection system at McNary Dam scheduled for fall 1984 was not completed on schedule because adequate numbers of tags were not available from the company. These tests have been rescheduled for spring 1985.

Conclusions and Recommendations

The initial testing of the prototype PIT tag detector system for juvenile salmonids produced results that exceeded expectations. The system functioned well under demanding weather conditions, and its accuracy met or exceeded design criteria. If the tests scheduled for McNary Dam during spring 1985 show similiar results, it would appear that a detector system could be in place and ready for testing at the collector dams by spring 1986. We recommend that the PIT tag development program continue as presently scheduled with this goal in mind.

PHASE III: MONITORING PIT TAGS IN ADULT FISH

Introduction

The PIT tag has significant potential for use in adults in two basically different type studies: (1) tags placed in smolts would result in data recovered at automatic monitors when the adult passes dams on its upstream spawning migration and (2) tags placed in adults at some point on their spawning migration and subsequent data recovered as in (1) above. The former use may replace current coded wire tagging (CWT) studies. The latter use would complement radio-tracking studies where research is needed on adult losses, migration delays, genetic stock identifications, and fall-back problems at dams or other migratory obstacles.

If the PIT tag is to have broad applicltion for research, detection and automatic data recording must be assured under a variety of field conditions. Therefore, our objectives were to: (1) evaluate the feasibility of monitoring PIT-tagged adult salmonids in a variety of situations applicable to Columbia River dams and (2) assess the accuracy and reliability of the PIT tag/detector system when used with adult salmonids.

Methods and Materials

A primary advantage seen for PIT tag applications is that fish carrying PIT tags need not be stopped or handled in any way to recover data. Therefore, our field trials in 1984 were designed to recover data (read tags) in moving fish.

Our trials were limited in scope because: (1) tags were not delivered by the company on schedule, (2) few functional tags were delivered, and (3) only one of several potential recovery situations could be tested. The trial situation chosen for monitoring the tag in adults was the trapping site used for monitoring coded wire tagged adults at dams. In this situation, fish ascend a denil fishway located in a normal fishway at dams, pass over a false weir, and finally slide downward through a CWT detector and if detected are shunted into a trap for observation.

We constructed a CWT trapping simulator at NMPS facilities at Pasco, Washington. The simple structure consisted of a 10 ft-long, 10 in-diameter fiberglass pipe placed at approximately a 30° angle downward. The pipe was connected to a horizontal pipe measuring 5 ft long and 12 in diameter. Four PIT tag detector coils were fabricated around the horizontal section of the pipe. The first and fourth coils were wrapped in vertical alignment with the pipe and the second and third were wrapped obliquely. Multiple detector loops and different loop orientations were used to increase the probability of tag detection given various tag orientations. The tag detection coils were connected to a coil excitor, controller assembly, and a recorder in a manner similar to that described in Phase 11 of this report (Fig. 1).

Trials were conducted at the Pasco facility in November 1984 with nine adult steelhead (57-84 cm fork length) and one chinook salmon jack (39 cm fork length). The fish were anesthetized and tagged internally according to methods described in Phase I of this report. After tagging, a hand-held detector was used to record tag numbers and to verify that each tag was functional.

When the adults were fully recovered from effects of anesthesia, they were dipnetted and placed into the entrance of the pipe. The fish were purposely not anesthetized to allow for maximum fish movement as would be expected in normal migrating fish passing through the monitor.

Results and Discussion

Results of field trials for automatic detection of PIT tags in adult salmonids are shown in Table 9. Detection for each trial day ranged from 91.0 to 97.1%. Even though our goal for these preliminary trials was 100% detection, the average detection of 94.4% was extremely encouraging.

Since availability of adults for test purposes was limited, each fish was reused with its original tag at least 20 times (only 10 tags were used). There were 13 instances of failure to detect. Of these, four were from the same tag. This indicates that either the tag was weak (reading distance short) or that placement in the fish was such that detection was difficult.

Our trials were designed to simulate recovery of tag data from adults having been previously tagged as juveniles (i.e., they were tagged internally). There may, however, be instances where the PIT tag may be used for adult information only. In this case, the fish could be tagged externally. We briefly explored this concept by using a plastic electrical wire tie as a jaw tag. The connecting portion of the tie was drilled to accommodate a PIT tag. The brief tests indicated that this tagging method might be useful in future studies with adult salmonids.

The installation and testing of the detection system at a dam, scheduled for fall 1984, was not conducted because functional tags were not available from the company. These tests have been rescheduled for summer 1985.

Conclusions and Recommendations

The initial testing of the prototype PIT tag detector system for adult salmonids was encouraging. Tag detection ranged from 91.0 to 97.1%

We believe, however, that detection in future studies will be increased by: (1) increased quality control in tag manufacture; (2) increased power

Table 9.--Detection of PIT tags placed in adult salmonids in simulated coded wire tag trapping facilities.

Date	Number of fish	Number of fish detected with tags	Detection (%)
19 November	100	91	91.0
29 November	41	39	95.1
30 November	70	68	97.1
Total	211	198	average 94.4

input through detection coils (3) using two detection systems in tandem, thus doubling the chance for detection; and (4) using an external tagging technique for adults so that the PIT tag orientation is in a Fixed position relative to the fish. We recommend that the PTT tag development program continue as scheduled.

ACKNOWLEDGMENTS

Support for this research came from the region's electrical ratepayers through the Bonneville Power Administration.

Special thanks are given to David Cross and Scott McCutcheon for their technical support.

LITERATURE CITED

Sokal, R. R. and F. J. Rohlf.
1981. Biometry. W. H. Freeman and Co., San Francisco, California.

APPENDIX

Budget Information

BUDGET SUMMARY

PHASES I, II, and III

A. Summary of expenditures

1.	Labor		$ 94.6K
2.	Travel		3.1K
3.	Supplies		191.4K
5.	NOAA and DOC overhead		38.3K
		TOTAL	$327.4K

B. Major property items (contracts)

1. PIT tag monitoring systems for juvenile and adult migrants--Contracts 84-ABC-00171 and 85-ABC-00134.

2. Components

   a. Four loop assemblies

   b. Four exciter assemblies

   c. Four controller assemblies

   d. Five printers

   e. Inter-connecting cabling